

PATENT COOPERATION TREATY

PCT



INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT-2757	FOR FURTHER ACTION		See Form PCT/IPEA/416
International application No. PCT/KR2005/000234	International filing date(day/month/year) 27 JANUARY 2005 (27.01.2005)	Priority date (day/month/year) 30 JANUARY 2004 (30.01.2004)	
International Patent Classification (IPC) or national classification and IPC C12N 9/24(2006.01)i, C12N 9/30(2006.01)i, C12N 15/56(2006.01)i, C12N 15/63(2006.01)i, C12N 9/00(2006.01)i			
Applicant LIFENZA CO., LTD. et al			

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of <u>4</u> sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising: a. <input checked="" type="checkbox"/> (sent to the applicant and to the International Bureau) a total of <u>4</u> sheets, as follows: <input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). <input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) _____ containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items: <input checked="" type="checkbox"/> Box No. I Basis of the report <input type="checkbox"/> Box No. II Priority <input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability <input type="checkbox"/> Box No. IV Lack of unity of invention <input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement <input type="checkbox"/> Box No. VI Certain documents cited <input type="checkbox"/> Box No. VII Certain defects in the international application <input type="checkbox"/> Box No. VIII Certain observations on the international application

Date of submission of the demand 24 AUGUST 2005 (24.08.2005)	Date of completion of this report 16 MAY 2006 (16.05.2006)
Name and mailing address of the IPEA/KR  Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer CHO, YOUNG GYUN  Telephone No. 82-42-481-8132

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

PCT/KR2005/000234

Box No. 1 Basis of the report

10/588140

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language _____ which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4)
- ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):
- ☐ the international application as originally filed/furnished
- ☒ the description:
- pages _____ 1-6, 8-18 _____ as originally filed/furnished
- pages* _____ 7 _____ received by this Authority on _____ 07/04/2006
- pages* _____ received by this Authority on _____
- ☒ the claims:
- pages _____ 20 _____ as originally filed/furnished
- pages* _____ as amended (together with any statement) under Article 19
- pages* _____ 19 _____ received by this Authority on _____ 07/04/2006
- pages* _____ received by this Authority on _____
- ☒ the drawings:
- pages _____ 1/6-6/6 _____ as originally filed/furnished
- pages* _____ received by this Authority on _____
- pages* _____ received by this Authority on _____
- ☒ the sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets _____
- ☐ the sequence listing (*specify*): _____
- ☐ any table(s) related to sequence listing (*specify*): _____
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets _____
- ☐ the sequence listing (*specify*): _____
- ☐ any table(s) related to sequence listing (*specify*): _____

* If item 4 applies, some or all of those sheets may be marked "superseded."

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-10	YES
	Claims	None	NO
Inventive step (IS)	Claims	1-10	YES
	Claims	None	NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims	None	NO

2. Citations and explanations (Rule 70.7)

The following documents have been considered for the purpose of this report:

D1: WO 2003/018790 A1 (LIFENZA CO., LTD.) 6 MARCH 2003

D2: WO 2001/066570 A1 (KIM et al.) 13 SEPTEMBER 2001

D3: J. Microbiol. Biotechnol., Vol. 9(3), pp. 260-264 (1999)

D4: Biosci. Biotechnol. Biochem., Vol. 64(2), pp. 223-228 (2000)

The present invention relates to an enzyme, having the amino acid sequence of SEQ. ID. NO:1, with the activity of hydrolyzing dextran, starch, mutan, inulin and levan; a gene (SEQ. ID. NO:2) encoding said enzyme; a transformed cell expressing said gene; a method of producing said enzyme; and a composition for the dextran removal and the plaque elimination.

D1-D4 disclose the DEXAMase (dextranase and amylase), having antiplaque and anticaries activities, having dextranase and amylase activities simultaneously and degrading insoluble glucans, from *Lipomyces starkeyi* KSM 22; a preparation method of DEXAMase; and an oral composition comprising the same.

However, none of the prior art documents disclose the amino acid sequence of the enzyme (SEQ. ID. NO:1) and the nucleotide sequence of gene (SEQ. ID. NO:2) encoding the enzyme, and said enzyme in this invention cannot be derived in an obvious manner from the prior art documents.

Therefore, claims 1-10 meet the requirements of novelty, inventive step and industrial applicability under PCT Article 33(2)-(4).

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

a. type of material

☒

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☒

on paper

☐

in electronic form

c. time of filing/furnishing

☐

contained in the international application as filed

☐

filed together with the international application in electronic form

☐

furnished subsequently to this Authority for the purposes of search and/or examination

☒

received by this Authority as an amendment* on 07/04/2006

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed of furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

(corresponding to U.S. Pat. No. 6,485,953 dated Nov. 26, 2002) which relates to a DXAMase enzyme capable of hydrolyzing both dextran and starch, a microorganism producing the enzyme (identified as *Lipomyces starkeyi* KFCC-11077), and a
5 - composition comprising the enzyme.

The enzyme expressed from the gene (*lsd1*) of the present invention is capable of hydrolyzing starch and mutan (insoluble glucan) as well as dextran. Also, the glycanase according to the present invention is found to degrade dextran
10 mainly into glucose, isomaltose and isomaltotriose, with the concurrent production of smaller amounts of branched pentaoses and hexaoses.

Both levan- and inulin-type fructans, which are constituents of dental plaque, can be degraded by the
15 glycanase according to the present invention.

Accordingly, effective degradation of glucans, whether soluble or insoluble, can be achieved by the glycanase of the present invention. As it can prevent the formation of plaque and remove previously formed plaque by inhibiting the
20 colonization of bacteria and the aggregation of glucans, the glycanase is useful in preventing tooth cavities. It is inferred that the glycanase has the ability to remain on the teeth as demonstrated by a test for whether or not the enzyme binds to hydroxyapatite which is similar to tooth enamel
25 components.

Also, the present invention is concerned with a novel microorganism carrying a gene encoding the glycanase. The microorganism, a *Saccharomyces cerevisiae* pYLSd1, was

WHAT IS CLAIMED IS:

1. A protein, comprising an amino acid sequence of SEQ. ID. No. 1, which has the activity of hydrolyzing dextran, starch, mutan, inulin and levan, a derivative thereof, or a fragment thereof.
2. A gene of SEQ. ID. No. 2, encoding the protein, the derivative, or the fragment of claim 1, a derivative thereof, or a fragment thereof.
3. A transformed cell, expressing the gene, the derivative, or the fragment of claim 2.
4. The transformed cell as defined in claim 3, wherein the cell is prokaryotic or eukaryotic.
5. The transformed cell as defined in claim 3 or 4, wherein the cell is *Saccharomyces cerevisiae* pYLSd1 deposited on Dec. 24, 2003, with the accession number KCTC 10574BP.
6. A method of producing an enzyme having activity of hydrolyzing dextran, starch, mutan, inulin and levan, comprising:
 - culturing the cell of claim 3;
 - expressing the enzyme in the cultured cell; and
 - purifying the expressed enzyme.

AMENDED SHEET (ART. 34)

【Sequence Listing】

<110> Lifenza Co., Ltd.

<120> PROTEIN WITH ACTIVITY OF HYDROLYZING DEXTRAN, STARCH, MUTAN,
5 INULIN AND LEVANN, GENE ENCODING THE SAME, CELL EXPRESSING THE
SAME, AND PRODUCTION METHOD THEREOF

<150> KR2004-0006185

<151> 2004-01-30

10 <160> 4

<170> KopatentIn 1.71

15 <210> 1
<211> 608
<212> PRT
<213> Artificial Sequence

20 <220>
<223> *Saccharomyces cerevisiae* pYLS01

<400> 1

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1 5 10 15

Thr Arg Ile Val Leu Val Asn Ile Leu Leu Ala Thr Leu Val Leu Gly
20 25 30

30 Ala Ala Val Leu Pro Arg Asp Asn Arg Thr Val Cys Gly Ser Gln Leu
35 40 45

Cys Thr Trp Trp His Asp Ser Gly Glu Ile Asn Thr Gly Thr Pro Val
35 50 55 60

Gln Ala Gly Asn Val Arg Gln Ser Arg Lys Tyr Ser Val His Val Ser

AMENDED SHEET (ART. 34)

Thr Gly Ile Ser Ile Asp Asn Leu His Val Ile His Thr Arg Tyr Phe
465 470 475 480

Lys Ser Glu Thr Val Val Pro Ser Ala Ile Ile Gly Ala Ser Pro Phe
5 485 490 495

Tyr Ala Ser Gly Met Thr Val Asp Pro Ser Glu Ser Ile Ser Met Thr
500 505 510

Ile Ser Asn Val Val Cys Glu Gly Leu Cys Pro Ser Leu Phe Arg Ile
10 515 520 525

Thr Pro Leu Gln Ser Tyr Asn Asn Leu Val Val Lys Asn Val Ala Phe
530 535 540

Pro Asp Gly Leu Gln Thr Asn Pro Ile Gly Ile Gly Glu Ser Ile Ile
15 545 550 555 560

Pro Ala Ala Ser Gly Cys Thr Met Asp Leu Glu Ile Thr Asn Trp Thr
20 565 570 575

Val Lys Gly Gln Lys Val Thr Met Gln Asn Phe Gln Ser Gly Ser Leu
580 585 590

Gly Gln Phe Asp Ile Asp Gly Ser Tyr Trp Gly Gln Trp Ser Ile Asn
25 595 600 605

30

<210> 2
<211> 2052
<212> DNA
<213> Artificial Sequence

35

<220>
<223> *Saccharomyces cerevisiae* pYLSd1